

Reisolation of plaques for cDNA cloning, sequencing and reconstruction of previously designed replicons and helpers



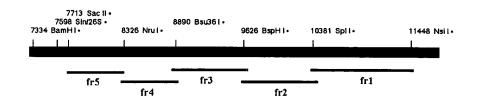




Fig. 2A

Figure 2B

ATTGACGGCGTAGTACACACTATTGAATCAAACAGCCGACCAATTGCACTACCATCACAATGGAGAAGCCAGTAG TAAACGTAGACGTAGACCCCCAGAGTCCGTTTGTCGTGCAACTGCAAAAAAGCTTCCCGCAATTTGAGGTAGTAG CACAGCAGGTCACTCCAAATGACCATGCTAATGCCAGAGCATTTTCGCATCTGGCCAGTAAACTAATCGAGCTGG AGGTTCCTACCACAGCGACGATCTTGGACATAGGCAGCGCACCGGCTCGTAGAATGTTTTCCGAGCACCAGTATC ATTGTGTCTGCCCCATGCGTAGTCCAGAAGACCCGGACCGCATGATGAAATATGCCAGTAAACTGGCGGAAAAAG CGTGCAAGATTACAAACAAGAACTTGCATGAGAAGATTAAGGATCTCCGGACCGTACTTGATACGCCGGATGCTG AAACACCATCGCTCTGCTTTCACAACGATGTTACCTGCAACATGCGTGCCGAATATTCCGTCATGCAGGACGTGT ATATCAACGCTCCCGGAACTATCTATCATCAGGCTATGAAAGGCGTGCGGACCCTGTACTGGATTGGCTTCGACA CCACCCAGTTCATGTTCTCGGCTATGGCAGGTTCGTACCCTGCGTACAACACCAACTGGGCCGACGAGAAAGTCC TTGAAGCGCGTAACATCGGACTTTGCAGCACAAAGCTGAGTGAAGGTAGGACAGGAAAATTGTCGATAATGAGGA ${f AGAAGGAGTTGAAGCCCGGGTCGCGGGTTTATTTCTCCGTAGGATCGACACTTTATCCAGAACACAGAGCCAGCT}$ TGCAGAGCTGGCATCTTCCATCGGTGTTCCACTTGAATGGAAAGCAGTCGTACACTTGCCGCTGTGATACAGTGG TGAGTTGCGAAGGCTACGTAGTGAAGAAATCACCATCAGTCCCGGGATCACGGGAGAAACCGTGGGATACGCGG TTACACACAATAGCGAGGGCTTCTTGCTATGCAAAGTTACTGACACAGTAAAAGGAGAACGGGTATCGTTCCCTG TGTGCACGTACATCCCGGCCACCATATGCGATCAGATGACTGGTATAATGGCCACGGATATATCACCTGACGATG AAAATTACCTTCTGCCGATCATAGCACAAGGGTTCAGCAAATGGGCTAAGGAGCGCAAGGATGATCTTGATAACG AGAAAATGCTGGGTACTAGAGAACGCAAGCTTACGTACGGCTGCTTGTGGGCCGTTTCGCACTAAGAAAGTACATT CGTTTTATCGCCCACCTGGAACGCAGCCATCGTAAAAGTCCCAGCCTCTTTTAGCGCTTTTCCCATGTCGTCCG TATGGACGACCTCTTTGCCCATGTCGCTGAGGCAGAAATTGAAACTGGCATTGCAACCAAAGAAGGAGGAAAAAC TGCTGCAGGTCTCGGAGGAATTAGTCATGGAGGCCAAGGCTGCTTTTGAGGATGCTCAGGAGGAAGCCAGAGCGG AGAAGCTCCGAGAAGCACTTCCACCATTAGTGGCAGACAAAGGCATCGAGGCAGCCGCAGAAGTTGTCTGCGAAG TGGAGGGGCTCCAGGCGGACATCGGAGCAGCATTAGTTGAAACCCCGCGCGGTCACGTAAGGATAATACCTCAAG CGCACCCGCTAGCAGATCAGGTTAAGATCATAACACACTCCGGAAGATCAGGAAGGTACGCGGTCGAACCATACG TGGAGGGACTGAAGACCCGACCTGCGGTCCCGTACAAGGTCGAAACAATAGGAGTGATAGGCACACCGGGGTCGG AAATTGAGGCCGACGTGCTAAGACTGAGGGGTATGCAGATTACGTCGAAGACAGTAGATTCGGTTATGCTCAACG GATGCCACAAAGCCGTAGAAGTGCTGTACGTTGACGAAGCGTTCGCGTGCCACGCAGGAGCACTACTTGCCTTGA TTGCTATCGTCAGGCCCCGCAAGAAGGTAGTACTATGCGGAGACCCCATGCAATGCGGATTCTTCAACATGATGC AACTAAAGGTACATTTCAATCACCCTGAAAAAGACATATGCACCAAGACATTCTACAAGTATATCTCCCGGCGTT GCACACAGCCAGTTACAGCTATTGTATCGACACTGCATTACGATGGAAAGATGAAAACCACGAACCCGTGCAAGA AGAACATTGAAATCGATATTACAGGGGCCACAAAGCCGAAGCCAGGGGATATCATCCTGACATGTTTCCGCGGGT GGGTTAAGCAATTGCAAATCGACTATCCCGGACATGAAGTAATGACAGCCGCGGCCTCACAAGGGCTAACCAGAA AAGGAGTGTATGCCGTCCGGCAAAAAGTCAATGAAAACCCACTGTACGCGATCACATCAGAGCATGTGAACGTGT TGCTCACCCGCACTGAGGACAGGCTAGTGTGGAAAACCTTGCAGGGCGACCCATGGATTAAGCAGCTCACTAACA TACCTAAAGGAAACTTTCAGGCTACTATAGAGGACTGGGAAGCTGAACACAAGGGAATAATTGCTGCAATAAACA GCCCCACTCCCCGTGCCAATCCGTTCAGCTGCAAGACCAACGTTTGCTGGGCGAAAGCATTGGAACCGATACTAG ${\tt CCACGGCCGGTATCGTACTTACCGGTTGCCAGTGGAGCGAACTGTTCCCACAGTTTGCGGATGACAAACCACATT}$ ${\tt CGGCCATTACGCCTTAGACGTAATTTGCATTAAGTTTTTCGGCATGGACTTGACAAGCGGACTGTTTTCTAAAC}$ GCACACAACTTGATTTGCAGACGGGGAGAACCAGAGTTATCTCTGCACAGCATAACCTGGTCCCGGTGAACCGCA ${ t ATCTTCCTCACGCCTTAGTCCCCGAGTACAAGGAGAAGCAACCCGGCCCGGTCGA}{ t AAATTCTTGAACCAGTTCA}$ AACACCACTCAGTACTTGTGGTATCAGAGGAAAAATTGAAGCTCCCCGTAAGAGAATCGAATGGATCGCCCCGA ${ t TTGGCATAGCCGGTGCAGATAAGAACTACAACCTGGCTTTCGGGTTTCCGCCGCAGGCACGGTACGACCTGGTGT}$ TCATCAACATTGGAACTAAATACAGAAACCACCACTTTCAGCAGTGCGAAGACCATGCGGCGACCTTAAAAACCC ${\tt TTTCGCGTTCGGCCCTGAATTGCCTTAACCCAGGAGGCACCCTCGTGGTGAAGTCCTATGGCTACGCCGACCGCA}$ ACAGTGAGGACGTAGTCACCGCTCTTGCCAGAAAGTTTGTCAGGGTGTCTGCAGCGAGACCAGATTGTGTCTCAA

GCAATACAGAAAT ACCTGATTTTCCGACAACTAGACAACAGCCGTACACGGCAATTCACCCCGCACCATCTGA ATTGCGTGATTTCGTCCGTGTATGAGGGTACAAGAGATGGAGTTGGAGCCGCGCCGTCATACCGCACCAAAAGGG A GAATATTGCTGACTGTCAAGAGGAAGCAGTTGTCAACGCAGCCAATCCGCTGGGTAGACCAGGCGAAGGAGTCTGCCGTGCCATCTATAAACGTTGGCCGACCAGTTTTACCGATTCAGCCACGGAGACAGGCACCGCAAGAATGACTG TGTGCCTAGGAAAGAAAGTGATCCACGCGGTCGGCCCTGATTTCCGGAAGCACCCCAGAAGCAGAAGCCTTGAAAT TGCTACAAAACGCCTACCATGCAGTGGCAGACTTAGTAAATGAACATAACATCAAGTCTGTCGCCATTCCACTGC TATCTACAGGCATTTACGCAGCCGGAAAAGACCGCCTTGAAGTATCACTTAACTGCTTGACAACCGCGCTAGACA GCTTGAAGGGAAGAAGGGATTCAGTACTACAAAAGGAAAATTGTATTCGTACTTCGAAGGCACCAAATTCCATC AAGCAGCAAAAGACATGGCGGAGATAAAGGTCCTGTTCCCTAATGACCAGGAAAGTAATGAACAACTGTGTGCCT AAACGTTGCCGTGCCTTTGCATGTATGCCATGACGCCAGAAAGGGTCCACAGACTTAGAAGCAATAACGTCAAAG AAGTTACAGTATGCTCCTCCACCCCCCTTCCTAAGCACAAAATTAAGAATGTTCAGAAGGTTCAGTGCACGAAAG CTCCTCCTGCACAGGCCGAGGGCCCCCGAAGTTGTAGCGACACCGTCACCATCTACAGCTGATAACACCTCGC TTGATGTCACAGACATCTCACTGGATATGGATGACAGTAGCGAAGGCTCACTTTTTTCGAGCTTTAGCGGATCGG ${\tt ACAACTCTATTACTAGTATGGACAGTTGGTCGTCAGGACCTAGTTCACTAGAGATAGTAGACCGAAGGCAGGTGG}$ TGGTGGCTGACGTTCATGCCGTCCAAGAGCCTGCCCCTATTCCACCGCCAAGGCTAAAGAAGATGGCCCGCCTGG CAGCGGCTAGAAAAGAGCCCACTCCACCGGCAAGCAATAGCTCTGAGTCCCTCCACCTCTCTTTTGGTGGGGTAT CGGATGTGCCTATGTCTTTCGGATCGTTTTCCGACGGAGAGATTGATGAGCTGAGCCGCAGAGTAACTGAGTCCG ${\tt AACCCGTCCTGTTTGGATCATTTGAACCGGGCGAAGTGAACTCAATTATATCGTCCCGATCAGCCGTATCTTTTC}$ CACTACGCAAGCAGAACGTAGACGCAGGAGCAGGAGGACTGAATACTGACTAACCGGGGTAGGTGGGTACATAT AGCGCAATGTCCTGGAAAGAATTCATGCCCCGGTGCTCGACACGTCGAAAGAGAAGAACAACTCAAACTCAGGTACC AGRIGATGCCCACCGAAGCAACAAAGTAGGTACCAGTCTCGTAAAGTAGAAAATCAGAAAGCCATAACCACTG AGCGACTACTGTCAGGACTACGACTGTATAACTCTGCCACAGATCAGCCAGAATGCTATAAGATCACCTATCCGA TGCATGAGAACTATCCGACAGTAGCATCTTATCAGATTACTGACGAGTACGATGCTTACTTGGATATGGTAGACG GGACAGTCGCCTGGATACTGCAACCTTCTGCCCCGCTAAGCTTAGAAGTTACCCGAAAAAACATGAGTATA GAGCCCCGAATATCCGCAGTGCGGTTCCATCAGCGATGCAGAACACGCTACAAAATGTGCTCATTGCCGCAACTA AAAGAAATTGCAACGTCACGCAGATGCGTGAACTGCCAACACTGGACTCAGCGACATTCAATGTCGAATGCTTTC GAAAATATGCATGTAATGACGAGTATTGGGAGGAGTTCGCTCGGAAGCCAATTAGGATTACCACTGAGTTTGTCA CCGCATATGTAGCTAGACTGAAAGGCCCTAAGGCCGCCGCACTATTTGCAAAGACGTATAATTTGGTCCCATTGC AAGAAGTGCCTATGGATAGATTCGTCATGGACATGAAAAGGGACGTGAAAGTTACACCAGGCACGAAACACACAG ${\tt AATTAGTGCGTAGGCTTACGGCCGTCTTGCTTCCAAACATTCACACGCTTTTTGACATGTCGGCGGAGGATTTTG}$ ATGCAATCATAGCAGAACACTTCAAGCAAGGCGACCCGGTACTGGAGACGGATATCGCATCATTCGACAAAAGCC AAGACGACGCTATGGCGTTAACCGGTCTGATGATCTTGGAGGACCTGGGTGTGGATCAACCACTACTCGACTTGA AATCCGGAATGTTCCTCACACTTTTTGTCAACACAGTTTTGAATGTCGTTATCGCCAGCAGAGTACTAGAAGAGC GGCTTAAAACGTCCAGATGTGCAGCGTTCATTGGCGACGACAACATCATACATGGAGTAGTATCTGACAAAGAAA TGGCTGAGAGGTGCGCCACCTGGCTCAACATGGAGGTTAAGATCATCGACGCAGTCATCGGTGAGAGACCACCTT ACTTCTGCGGCGGATTTATCTTGCAAGATTCGGTTACTTCCACAGCGTGCCGCGTGGCGGACCCcctgaaaaggc tgtttaagttgggtaaaccgctcccagccgacgacgagcaagacgaagacagaagacgcgctctgctagatgaaa caaaggcgtggtttagagtaggtataacaggcactttagcagtggccgtgacgacccggtatgaggtagacaata ttacacctgtcctactggcattgagaacttttgcccagagcaaaagagcattccaagccatcagaggggaaataa agcatetetacggtggteetaaatagteagcatagtacattteatetgaetaataetaeaacaecaecatga GGCAGGCGGCCCGATGCCCGCAACGGGCTGGCTTCTCAAATCCAGCAACTGACCACAGCCGTCAGTGCCC AGAGACAGCGCATGGCACTTAAGTTGGAGGCCGACAGATCGTTCGACGTCAAGAACGAGGACGGAGATGTCATCG GGCACGCACTGGCCATGGAAGGAAAGGTAATGAAACCTCTGCACGTGAAAGGAACCATCGACCACCCTGTGCTAT ${ t CAAAGCTCAAATTTACCAAGTCGTCAGCATACGACATGGAGTTCGCACAGTTGCCAGTCAACATGAGAAGTGAGG}$ CATTCACCTACACCAGTGAACACCCCGAAGGATTCTATAACTGGCACCACGGAGCGGTGCAGTATAGTGGAGGTA

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Figure 2C

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Figure 3. Infection of human dendritic cells with a DC adapted alphavirus vector (DC+) expressing GFP

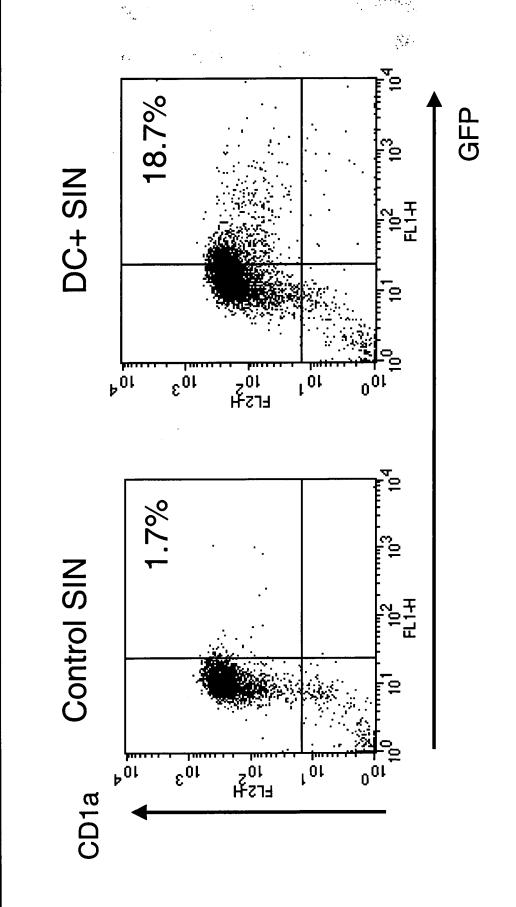


Figure 4. Increased potency of new SINCR alphavirus replicon

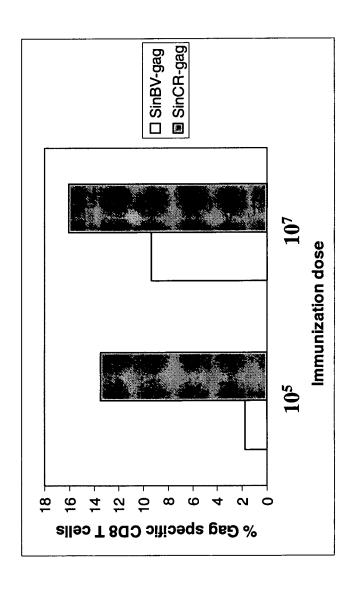
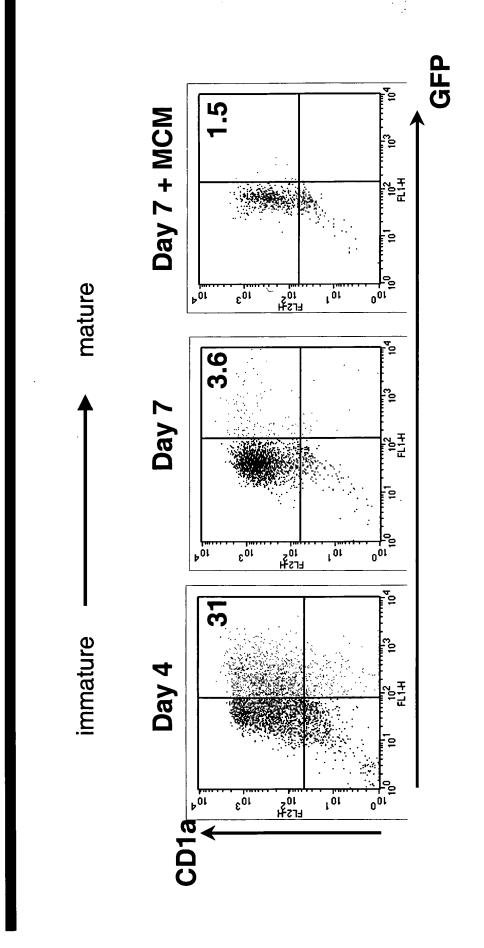
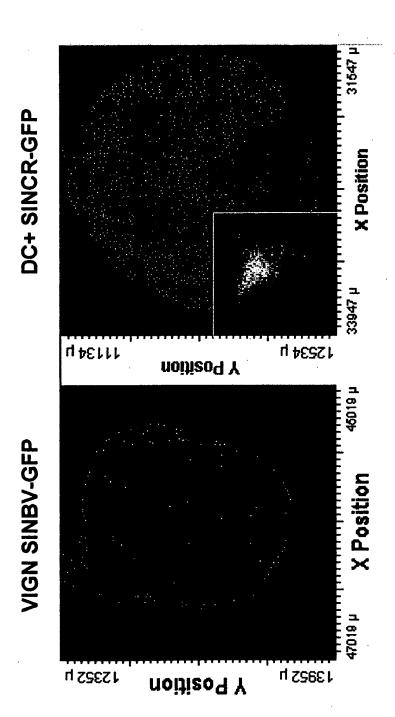


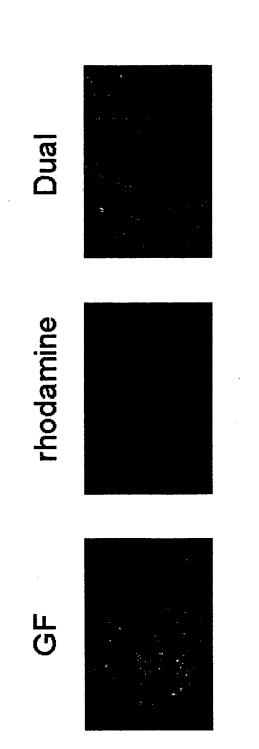
Figure 5. DC+ SIN vectors target immature human dendritic cells





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Figure 7. Trafficking of alphavirus vector transduced DC to the mandibular lymph node

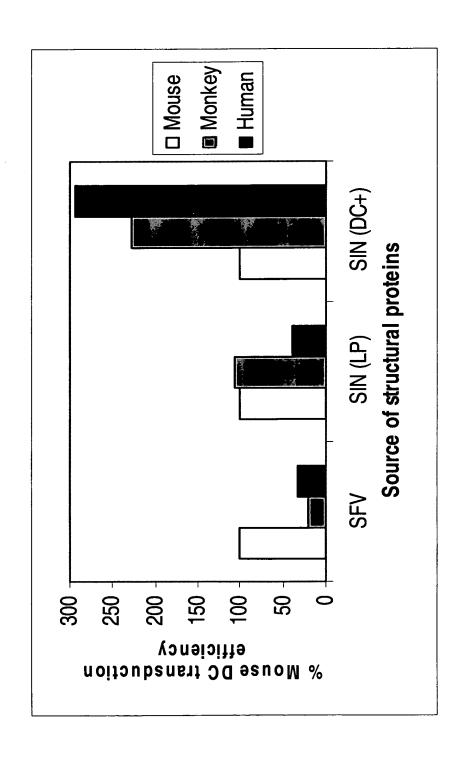


SIN-GFP vector injected intradermally, with rhodamine paint applied to skin

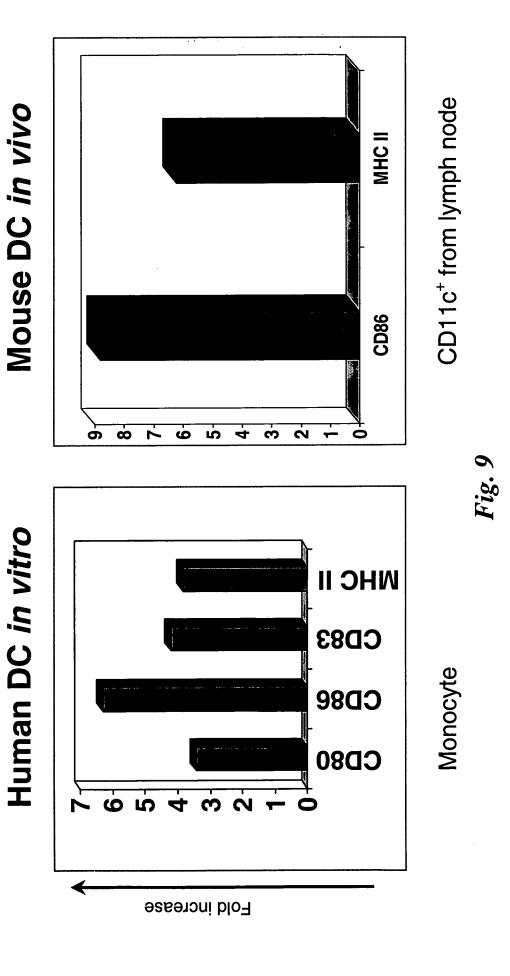
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Figure 8. Mouse DC transduction is not predictive of the ability of alphavirus vectors to transduce human DC



Alphavirus vectors can induce DC maturation and activation both in vitro and in vivo



Adapted alphavirus vectors can be used to assay antigen presentation and immune stimulation in vitro

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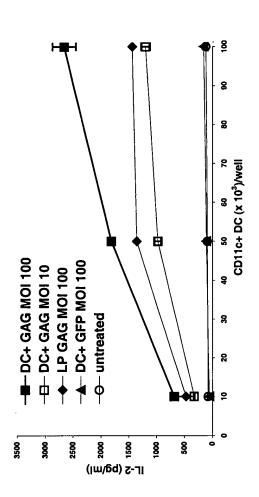


Fig. 16

Increased potency of new SINCR alphavirus replicon

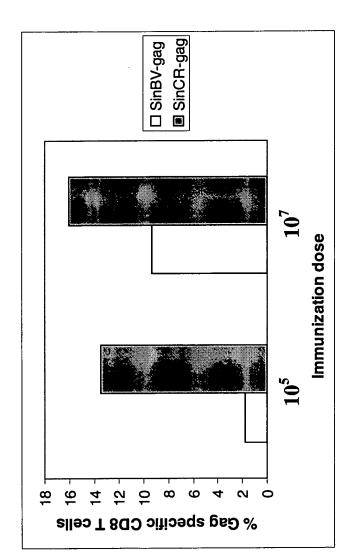


Fig. 11

Enhanced immune response by using a prime-boost strategy

